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Presence of Hemoglobinopathies in Sicily: A Historic Perspective

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Sicily, at the center of the Mediterranean, has been the meeting place of Eastern and Western civilizations, and in the Sicilian population the presence of many different alterations in the globin gene clusters can surely be considered testimony of past colonizations. From 1975 to 1994, 100,000 Sicilian subjects were screened by us to monitor the presence of hemoglobin (Hb) structural variants. In this paper we present the data gathered, emphasizing the high incidence (2.5%) of carriers of at least one abnormal Hb, and the great heterogeneity of globin molecular defects on the island. Twenty-six different mutations were identified: the most common occur in the β-globin gene (β^S, β^C, $\delta\beta^{Lepore}$, $\beta^{G\text{-San José}}$, $\beta^{O\text{-Arab}}$), but also quite frequent is the mutated allele $\alpha^{J\text{-Oxford}}$. The chromosome haplotypes associated with some of them were characterized. Two uncommon Hbs, Copenhagen and D Punjab, and some 18 rare variants complete the wide spectrum of structural alterations of globin genes in Sicily. We think they are de novo mutations prevalently. It is not possible to exclude that the presence of a few of them is related to migratory phenomena, particularly from North Africa and East Asia. Numerous thalassemic alleles complete the picture of globin gene mutations in Silicy. Am. J. Med. Genet. 69: 200–206, 1997.

KEY WORDS: hemoglobin variants; thalassemic mutations; hemoglobinopathies

"Doch in Sizilien findet sich der Schlussel für alles"

J.W. Goethe

INTRODUCTION

Sicily is the largest island in the Mediterranean (25.126 km^2), with a population of 4.9 million. Its individuality, which has been maintained even though it is only 3 km from the Italian peninsula (Straits of Messina), has favored a historic development characterized by originality in costume, art, and culture. Due to its geographic position (the island is almost in the middle of the Mediterranean), it has for centuries been the center of the "Mediterranean continent." In different times the necessity of finding new commercial routes, together with a taste for adventure and fascination of the unknown, has always been the motive for the Mediterranean peoples to make new voyages of exploration, favored and encouraged by climatic and morphologic uniformity of the Mediterranean coastline. In this context, Sicily has been the landing place of ancient civilizations, where different cultures met. Archaeological remains and important monuments have been found throughout the island and testify to the sequential passage of Greeks and Phoenicians, Etruscans and Romans, Byzantines and Arabs, Normans, Aragons, and Bourbons. It is above all in the genetic structure of the population that the information of its historic evolution is stored. The heterogeneity of the anthropometric characteristics in the Sicilians (eye color, hair color, stature, etc.) seems to reflect the complex historical events that have swept through the island in the last few millennia. And again, as a result of this genetic admixture, many different anomalies in the globin gene clusters have been found. In this study, we report the structural hemoglobin (Hb) variants identified in Eastern Sicily during a thalassemia screening program carried out by our Division from 1975 to 1994 and we try to summarize the knowledge of their origin and distribution on the island.

MATERIALS AND METHODS

Subjects

During 20 years of activity (1975–1994) in our Division of Pediatric Hematology and Oncology at the University of Catania, 100,000 subjects, largely from Eastern Sicily, have been characterized. They constitute a group heterogeneous in age (range from 1 to 65 years;

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average 30 years), socioeconomic conditions, and cultural level. Generally, they seemed clinically healthy, and the presence of at least one globin mutation in several of them has not given rise to any clinical expression. The subjects in which a Hb variant was coinherited with a β-thalassemic allele (or other globin variant) have shown variable clinical severity and different hematological pictures.

In most cases, the subjects came to our attention via the family doctor, pediatrician, or gynecologist. The widespread information campaign, which has been extended to schools, marriage guidance councelors, and healthy blood donors had important effects. Quite often, subjects having undergone a medical examination and hematological investigation were found to be heterozygotes for a Hb variant; usually family studies were essential to shed light on Hb and hematological conditions in children hospitalized for acute illness or hematological problems. In addition, we studied 403 subjects with thalassemia major, 44 with thalassemia intermedia, 100 compound heterozygotes for Hb S-β thal, 33 homozygotes for Hb S, and 15 patients with Hb H disease. In these subjects, the diagnosis was based on clinical evaluation, hematological analysis, and genetic studies.

One hundred and seventy-nine newborn babies (aged 0–2 months) were analyzed in search of abnormal fetal Hbs.

Methods

About 5 ml of venous blood with EDTA as anticoagulant (10 mM) was sufficient for the determination of the hematological parameters and fundamental Hbs. The red cell indices were evaluated with an automatic Coulter Counter Cell. Blood films allowed the observation of the red cell morphology. Hb A_2 was quantified by microchromatography on DEAE-cellulose. Separation of the Hb types was obtained by electrophoresis (electrophoresis on cellulose acetate and citrate agar) and ion exchange high performance liquid chromatography (HPLC) [Samperi et al., 1991]. The HPLC analysis gave quantitative data. Red cell lysates were also analyzed by a reversed phase HPLC procedure to evaluate the relative quantities of globin chains [Kutlar et al., 1986]. The samples in which we suspected the presence of Hb S underwent a sickle test with 2% sodium metabisulphate.

Aliquots of blood were kept at 4°C for various periods of time (1–5 days and then shipped in ice by post carrier services to Augusta, GA). High molecular weight DNA was isolated from peripheral blood leukocytes following the protocol reported by Poncz et al. [1982]. The analysis of polymerase chain reaction (PCR)-amplified DNA was performed using ^{32}P-labeled oligonucleotides probes [Dimovski et al., 1990]. A few samples were studied by direct sequencing of amplified DNA [Dimovski et al., 1994]. The haplotype analysis in the β-globin cluster was performed by following restriction enzymes: Hinc II 5′ to ϵ, Xmn I 5′ to $^{G}\gamma$, Hind III at $^{G}\gamma$ and $^{A}\gamma$, Hinc II at ψβ and 3′ to it, Ava II at β, and Bam HI 3′ to β.

RESULTS

Hb Variants

Hitherto, 2,522 out of 100,000 individuals studied (2.5%) were found to be carriers of at least one structural Hb variant. Heterozygotes constituted the majority (91.6%), but the percentage of genetic compounds (5.6%), double heterozygotes (1.5%) and homozygotes (1.3%) is not negligible. Subjects who have coinherited a β-globin variant and a β-thal allele (118 cases) prevail among the compound heterozygotes, whereas the homozygous condition seems to concern almost exclusively, the falcemic mutation (33 in this survey), with the only exception for one CC individual.

We identified 26 different mutations; 17 of these alter the structure of the β-globin gene, 5 the δ-globin gene, while only 4 the α-globin gene. Table I shows the wide range of genetic combinations that were found. Based on their frequencies, some structural variants can be considered part of the genetic patrimony of the Sicilian population. As stated, the most common β variant is Hb S [β6(A3)Glu→Val]. It has often been observed in association with other abnormal β or α-globin variants. The 1,725 cases mentioned in this report include 33 subjects with homozygosity for Hb S, 70 with Hb S/β^0-thal, and 34 with Hb S/β^+-thal, 2 patients with the rare Hb S/$\delta\beta^{Lepore}$ condition, one with Hb S/δβ-thal [Mirabile et al., 1995], and a double heterozygote with β^S and $\alpha^{J\text{-}Oxford}$ alleles. What is striking in these patients is the clinical variability.

Molecular characterization of the β^S chromosomes in 81 patients with Hb S/β-thal, 23 patients with SS, and 31 heterozygous subjects showed a chromosomal background with the Benin haplotype. In only one instance was the uncommon Cameroon haplotype discovered [Schiliro' et al. 1992]. We also observed with a certain frequency Hb Lepore-Boston, Hb C, Hb G-San José, and, among the α variants, Hb J-Oxford.

Of 100,000 subjects screened, 235 were found to be heterozygotes for Hb Lepore. DNA analysis confirmed that all subjects carried the hybrid δβ-globin genes of the Boston-Washington type ($\delta^{87}\beta^{116}$).

We found that Hb J-Oxford [α15(A13)Gly→Asp] occurred at a similar frequency. The 231 carriers of $\alpha^{J\text{-}Oxford}$ are from 93 unrelated families, and come from small villages located around Catania. Most are simple heterozygotes (196 cases), who are clinically and hematologically normal. In addition, we found 34 double heterozygotes who, apart from the α gene, have a β-thal mutation. We also report a case of homozygous β^0-thal and $\alpha^{J\text{-}Oxford}$ and 6 subjects with a compound heterozygosity for α2 thal/Hb J-Oxford.

Hb C [β6(A3)Glu→Lys] is the fourth most common variant. We have identified 154 subjects with the β^C mutation, among them two unusual patients with CC and SC genotype, respectively. The β^C allele appears to be associated with two different haplotypes (I and II). Haplotype I, the most frequent, lacks an Hpa I recognition site, localized 5 kb 3′ to the β-globin gene and is associated with the restriction fragment of 13.0 kb. Haplotype II is less common and is characterized in Sicilian patients by the presence of an Hpa I cleavage site that produces an atypical 7.0 kb restriction fragment [Travi et al., 1992].

The Hb G-San José [β7(A4)Glu→Gly] mutation, even if appearing regularly in our samples, was found to be restricted to an area about 50 km from Catania, which

TABLE I. Human Hb Variants Identified in the Sicilian Population

Genotype	No. of cases	Structural alteration	Genetic combinations found
Hb S	1,725	β^{6}Glu→Val	S-S S-A S-β^{0}th S-β^{+}th S-J Oxford S-δβth
Hb J-Oxford	231	α^{15}Gly→Asp	J-β^{0}th J-A J-S
Hb C	154	β^{6} Glu→Lys	C-A C-β^{0}th C-β^{+}th C-C C-S
Hb Lepore Boston	235	δβ fusion(δ^{87} β^{116})	Lep-A Lep-β^{0}th Lep-β^{+}th Lep-HPFH Lep-δβ th Lep-S
Hb G-San José	70	β^{7} Glu→Gly	G-αth G-β^{0}th G-β^{+}th G-A
Hb J-Baltimore	9	β^{16}Gly→Asp	J-β^{+}th J-A
Hb Sheperds Bush	11	β^{74}Gly→Asp	SB-A
Hb G-Copenhagen	8	β^{47}Asp→Asn	G-A
Hb O-Arab	24	β^{121}Glu→Lys	O-A O-β^{0}th
Hb D-Punjab	10	β^{121}Glu→Gln	D-A D-S
Hb Setif	3	α^{94}Asp→Tyr	Set-A
Hb Agenogi	8	β^{90}Glu→Lys	Ag-A Ag-β^{0}th
Hb G-Waimanalo	2	α^{64}Asp→Asn	W-A
Hb Camperdown	2	β^{104}Arg→Ser	Ca-A
Hb A_2' (B_2)	5	δ^{16}Gly→Arg	B_2-A B_2-ββ^{+}th
Hb G-Szuhu	3	β^{80} Asn→Lys	G-A
Hb Pyrgos	2	β^{83} Gly→Asp	P-A
Hb A_2NYU	3	δ^{12}Asn→Lys	A_2NY-A
Hb Malmö	3	β^{97} His→Gln	Malmö-A Malmö-β^{0}th
Hb Hope	2	β^{136}Gly→Asp	Hope-A
Hb A_2 Fitzroy	2	δ^{142}Ala→Asp	A_2 Fitzroy-A
Hb A_2 Coburg	2	δ^{116} Arg→His	A_2 Coburg-A
Hb E	2	β^{26} Glu→Lys	E-A
Hb Dhonburi	2	β^{126}Val→Gly	Dhonburi-A Dhonburi-β^{0}th
Hb Athens-Georgia	2	β^{40}Arg→Lys	Athens-A
Hb Constant Spring	2	α^{141} extended chain	Constant Spring-A Constant Spring-α^{0}th

includes the small town of Grammichele. The 70 subjects discovered in 15 apparently unrelated families come directly, or originate from the village. The study of the chromosomal background associated with this mutation has shown that there is a close linkage between the $\beta^{G\text{-San José}}$ allele and the Mediterranean haplotype IV [Cremonesi et al., 1989].

Among others, Hb O-Arab [β121(GH4)Glu→Lys] is a rather frequent mutation. This Hb was identified in 24 individuals from 7 different unrelated families, all coming from the province of Siracusa.

Hbs Copenhagen [β47(CD6)Asp→Asn] and D-Punjab [β121(GH4)Glu→Gln] were identified in different and apparently unrelated families. In addition, we have observed the presence of 18 rare variants (Table I), all in single families. In each family group, no less than two subjects were found to be carriers. This is the case for Hb Setif [α94(G1)Asp→Tyr], Hb G-Waimanalo [α64(E13) Asp→Asn], Hb Pyrgos [β83(EF7)Gly→Asp], Hb A_2' (B_2) [δ16(A13)Gly→Arg], Hb E [β26(B8)Glu→Lys], Hb Agenogi [β90(F6)Glu→Lys], and Hb G-Szuhu [β80(EF4) Asn→Lys]. Simple heterozygotes do not have either clinical manifestations or changes in hematological parameters. This is true for α, and δ variants and for most of the β variants. We also observed rare cases of double or compound heterozygosity (Table I), same of them not reported previously, such as Hb Agenogi/β^{thal}. It is not surprising that, due to their high prevalence (5.9%), the thalassemic mutations (β^{+} or β^{0}) are coinherited with these structural variants. Consequently the clinical and hematological picture of such compound heterozygotes can be quite variable.

β-Thalassemia

We have identified 15 different mutations of the total 846 chromosomes studied. The most common ones give rise to a nonfunctional mRNA (CD 39, C→T), or affect RNA processing [IVS-I-110(G→A); IVS-I-6(T→C);IVS-I-1(G→A),IVS-II-745(C→G)]. These mutations account for 90% of all β-thal alleles (Table II), and have comparable distributions all over Sicily. Rarer are the transcriptional mutants [−87(C→G); −92(C→T)], and the frameshift mutations [CD 5(−CT); CD 6(−A); CD 8(−AA)]. The chromosomal backgrounds associated with these different β-thal alleles in the Sicilian population are studied both in compound heterozygotes (169) patients and homozygous (98) patients. Unlike what has been observed in other Mediterranean regions, each of five mutations may be seen within few haplotypes (CD 39: haplotypes I and II; IVS-I-110: haplotype I; IVS-I-6: haplotypes VI and VII; IVS-I-1: haplotypes II and V; IVS-II-745: haplotype VII) [Schilirò et al., 1995].

α-Thalassemia

We discovered 15 patients with Hb H disease and determined the molecular basis of the genetic disorder. In all instances, family studies showed that one parent was an asymptomatic carrier of α-thal 2, whereas the other showed the typical red cell abnormalities of an α-thal 1 trait.

DNA analysis demonstrated that all 15 patients carried one chromosome without the two α-globin genes. This deletion was type $--^{MED}$, the most common in the Mediterranean area. In 11 patients, the inactivation of a third α-globin gene was also produced by a deletion, whereas, more rarely, we have observed a functional inactivation (only 4 patients in present series). In 10 subjects the $--^{MED}$ mutation was in combination with the $-\alpha^{3.7}$ allele (rightward deletion), and in one subject in association with the $-\alpha^{4.2}$ allele (leftward deletion). In one subject the nonexpression of one of two residual α

TABLE II. Type, Frequency, and Associated Haplotypes of Five Most Common β Thalassemic Genes in Sicily

Mutation	Type	No. chromosome studied	Frequency %	Associated haplotype
CD 39 (C→T)	β^0	292	34.52	I II
IVS-I-110 (G→A)	β^+	204	24.11	I
IVS-I-6 (T→C)	β^+	130	15.37	VI VII
IVS-I-1 (G→A)	β^0	76	9.00	II V
IVS-II-745 (C→G)	β^+	60	7.10	VII
Total		762	90.10	

genes was due to a single nucleotide substitution in the initiation codon of the α2 gene (A*T*G→A*C*G), changing the recognition site for the Nco I restriction enzyme. Another young patient showed the presence of an α chain termination mutant with 31 additional residues: Hb Constant Spring. In two cases it was not possible to determine the α-thal determinants (Table III).

γ-Globin Gene Anomalies

Preliminary studies of γ mutations in 179 newborn Sicilian babies suggest that the $^{A}\gamma$-Sardinia chain, or $^{A}\gamma^{T}$ chain, is the most frequent variant. We identified 5 $^{A}\gamma^{T}$ homozygotes (2.8%), and 49 $^{A}\gamma^{T}$ heterozygotes (27.3%). The $^{A}\gamma^{T}$ chain, quantified by reversed phase HPLC, elutes between the α and $^{G}\gamma$ chains. In the homozygous subjects, the percentage of $^{A}\gamma^{T}$ ranged from 25.5% to 31.9% (mean value: 28.6 ± 2.4%), whereas in the heterozygotes, the values varied from 11.6% to 16.8% (mean value: 14.6 ± 0.9%). The $^{G}\gamma$ levels, in the presence of the $^{A}\gamma^{T}$ chain are similar to those we have observed in the $^{A}\gamma^{T}$-negative condition (Table IV).

Four babies had unusually high levels of $^{G}\gamma$ (>80%), with high $^{G}\gamma/^{A}\gamma$ ratios (mean value 5.18), and average levels of $^{A}\gamma$ and β^{A}, of 16.2% and 22.6%, respectively. Finally, 2 newborn infants had low $^{G}\gamma$ levels (41.0, and 33.5%), with 29.2% and 21.2% Hb A_1, respectively. Molecular investigations are in progress to identify the probable γ-globin gene rearrangements.

DISCUSSION

Here we summarize data gathered during 20 years of thalassemia and Hb screening in Eastern Sicily. We think that, due to the large number of subjects studied, representing a vast area of the entire island, this survey, though limited to the characterization of globin gene clusters, provides useful information on the complex genetic structure of the Sicilian population. The numerous variants are proof of century-old contacts with other peoples and confirm that Sicily can be considered, at the same time, a country of African, Oriental, and Mediterranean civilization. The same area of diffusion of some structural variants seems to reflect the historic orientation of the southeast portion of the island towards the Italian Peninsula and Greece, and the western part towards Africa and Spain. This could be the case for HB S, which, contrary to what was observed for the β-thal mutation, is not uniformly distributed in the island, but with a prevalence along the southeastern coastal zone according to a north-south gradient, while it seems not be present in the western area [Barrai et al., 1987]. Probably the β^S allele arrived in Sicily, and in the Mediterranean area in general, when thalassemia was already present and uniformly distributed. We should also mention the small town of Butera, founded by the Arabs to the north of the Plain of Gela, where the frequency of the Hb S gene reaches 13%, while the β-thal mutations are present with the same frequency in the rest of Sicily (5.9%). The hypothesis of a de novo mutation has been ruled out due to the lack of the expected decrease that should happen in a ring-like fashion around the town [Barrai et al., 1987]. The explanation is probably found in the theoretic models of interaction between two alleles for anomalous Hb in a population [Livingstone, 1967; Cavalli-Sforza and Bodmer, 1972]. Molecular investigations showed the association of the sickle gene with specific haplotypes in three different regions of Africa: Benin, neighboring Nigeria, and Algeria, Central African Republic, and Senegal [Pagnier et al., 1984]. The Benin haplotype is prevalent in the Mediterranean populations (Greeks, Turks, Syrians, Tunisians, Arabs) and has been shown to be almost exclusive in the Sicilians. We have found in one case an uncommon type, the Cameroon haplotype, never seen before in Sicily [Schilirò et al., 1992]. Recently, the evaluation of genetic variability of the β-globin cluster in the Sicilian population was extended to polymorphic repetitive DNA segments in the hypersensitive site-2 (HS-2) of the locus control region (LCR). In contrast to the remarkable heterogeneity observed in normal individuals, our study confirms the presence of the β^S mutation on a single chromosome background (Labie D., unpublished data). These data suggest that the sickle gene, originating in Africa following recurrent mutations, diffused from West to North Africa through the trans-Saharan population migration and arrived in Sicily directly from North Africa.

TABLE III. Mutations in the α-Globin Gene Cluster Identified in 15 Hb H Sicilian Patients

Mutation	Type	No. of chromosomes
Deletion defects		
$--^{MED}$	α^0	15
$-\alpha^{3.7}$	α^+	10
$-\alpha^{4.2}$	α^+	1
Nondeletion defects		
$\alpha_2{}^{Nco\,I}\alpha_1$	α^+	1
$(\alpha\alpha)^{thal}$[a]	α^+	2
Hb Constant Spring	α^+	1

[a] Not yet characterized.

TABLE IV. The Relative Quantities of γ-Globin Chains From 179 Normal, $^{A}\gamma^{T}$ Heterozygous, and $^{A}\gamma^{T}$Homozygous Newborn Babies

Condition	No. of cases	Percentage[a]			
		$^{A}\gamma^{T}$	$^{G}\gamma$	$^{A}\gamma^{I}$	$^{G}\gamma/^{A}\gamma$
$^{A}\gamma^{T}$ negative	125	0	69.0 ± 5.4	30.7 ± 4.6	2.4 ± 0.6
$^{A}\gamma^{T}$ heterozygote	49	14.6 ± 0.9	69.3 ± 4.2	16.1 ± 3.9	2.3 ± 0.3
$^{A}\gamma^{T}$ homozygote	5	28.6 ± 2.4	71.4 ± 2.4	0	2.5 ± 0.3

[a] Average values and standard deviations.

The β^{c} mutation also arose in Africa [Trabuchet et al., 1991]. Particularly common in Western Africa and among Black Americans, it is also found, but with lower frequency, among the population of North Africa (greater than 1% in Morocco, 0.36% in Algeria) and, occasionally, in individuals originating from Sicily [Trabuchet et al., 1991; Schilirò, 1986]. The geographical distribution and the prevalent association of the β^{c} allele to a single haplotype both in Black American patients and in Africans have allowed the establishment of the unicentric origin of the mutation in Western Africa and its distribution in the populations of North Africa following gene migration. The history of slavery explains the introduction of this allele into North and Central America. In Sicily, the mutation appears linked to two haplotypes (I and II), previously described in Black Americans, and not common among the populations of the Mediterranean Basin. In Italy, they are rarely found in Sardinia and the southern regions, associated with normal alleles and β-thal [Travi et al., 1992]. It is therefore probable that the β^{c} mutation found in Sicily, as has already been hypothesized for β^{s}, originated in Africa.

Hb Lepore can also be considered a genetic contribution of past colonizers. This type of fusion protein, even if it has been found sporadically throughout the world and not confined to a single ethnic group, is more common in the Central and Eastern Mediterranean countries [Efremov, 1978]. The geographical distribution suggests that its occurrence can be correlated to the vast expansion of colonization by the Greeks up to 500 BC. In Italy, Hb Lepore-Boston is particularly present in the same southern regions (Campania, Calabria, and Sicily) that made up Magna Grecia and that for this mutation, malaria acted as a selection factor [Marinucci et al., 1979]. On the other hand, in regions such as Sardinia or the Po Delta, where the frequency of thalassemia is high but there is no trace of Greek settlements, the Lepore mutation is practically absent. It should be noted that the association of Hb Lepore-Boston with different haplotypes has recently suggested the possible independent multicentric origin of this mutation in Italy [Fioretti et al., 1992].

A different origin could explain the presence of Hb G-San José and Hb J-Oxford. The first is a rare β-globin variant, identified for the first time in West Africa. It was then found in a Mexican family, in individuals who originated from Calabria and, above all, in Sicilians [Edington and Lehmann, 1954; Schilirò al., 1981]. The distribution of the mutation is peculiar on our island; a large cluster was found in a restricted area that includes the town of Grammichele, only a few km from Catania. History can help to explain the genetic peculiarity of this town. The devastating earthquake of 1693 destroyed the town and killed most of the inhabitants. When the town was rebuilt, there followed a questionable "policy of repopulation." The small town was transformed into a refuge for criminals and fugitives from a law passed by Charles II of Austria. The almost total absence of subsequent immigration and, therefore, the genetic isolation of the population with consequent high frequency of consanguineous marriages (from 0.39% between 1780 and 1785, to 9–11% of all marriages between 1918 and 1924) could explain this cluster of Hb G-San José by "founder effect" [Schilirò and Li Volti, 1986]. The mutation is always associated with Mediterranean haplotype IV which is not frequently found in the Italian population, both in the normal (3.6%) and in β-thal chromosomes (0.6%). Perhaps the mutated $\beta^{\text{G-San José}}$ allele originated in Eastern Sicily following a single mutation event which occurred on a normal chromosome with haploptye IV [Cremonesi et al., 1989]. Its occasional presence in Calabria, a region at the extreme south of the Italian Peninsula, separated from Sicily only by the Straits of Messina, could be the result of successive migrations.

Hb J-Oxford is the most common α structural variant in Sicily. The cases so far reported refer to families from Southern Italy (Calabria, Campania, and Sicily). The relatively high frequency in a restricted area around Catania makes us believe that there is an independent origin for the mutation on the island [Schilirò et al., 1989].

Most Hbs shown listed in Table I can be considered rare variants. The presence of many in Sicily could be the consequence of independent mutations, because all were observed sporadically and the cases previously described are found in different racial groups. However, it is possible that the presence of some Hb variants is directly related to the historic function of Sicily, namely as a crossroad for commercial traffic and a landing place for people from all over the Old World. This could particularly be true for some Hbs such as Hb A_2' (B_2) (origin: Africa), Hb Setif (origin: Algeria, Saudi Arabia, Lebanon, Iran, Cyprus) [Schilirò et al., 1991; Chami et al., 1994], Hb Camperdown (origin: Malta) [Chami et al., 1994], Hb D Punjab (origin: Middle East Asia; Europe; North Africa) [Malcorra-Azpiazu et al., 1990].

In the last few years, widespread immigration has brought millions of non-Europeans (above all North Africans and East Asians) to Italy, and particularly to Sicily. This has further enriched the number of Hb mu-

tations present on the island. The presence of Hbs E and G-Waimanalo should be ascribed to this phenomenon [Schilirò et al., 1991].

The thalassemia mutations in Sicily represent the most common genetic disorders that occur in the globin genes. The defects in the expression of β-globin gene have been observed at a frequency of 5.9%, and we identified no less than 15 different β-thal alleles. The most frequent mutations, CD 39 and IVS-I-110, appear to be uniformly distributed [Schilirò et al., 1995], unlike other areas of the Mediterranean, where to the west (Spain, Morocco, Sardinia, Portugal, Algeria, Tunisia) the CD 39 mutation prevails, and to the east (Turkey, Greece, Yugoslavia, Cyprus) the IVS-I-110 mutation is prevalent [Cao et al., 1989; Amselem et al., 1988]. The association of the most frequent mutations with one or two haplotypes suggests that the introduction of the β-thal mutation happened relatively recently, with no recombination event or gene conversion having had time to take place. The high frequency of mutations which seriously compromises the expression of the β-globin gene explains the high percentage of patients with thalassemia major. Only 19% of our patients present a clinically less severe form, recognizable by the presence, in homozygotes or compound heterozygotes, of less severe mutations. The possibility of tracing 90% of the β-thal mutations to five types is of fundamental importance for conducting a prenatal diagnosis program.

The α-thal appears to occur at low frequency in Sicily; only 15 patients with Hb H disease have been identified. According to molecular studies in 250 newborn babies, α-thal 2 is present with a frequency of 0.012 and the 3.7 kb deletion seems prevalent [Fei et al., 1989]. DNA analyses in subjects with Hb H disease have indicated some molecular heterogeneity in the α-thal: in fact, $--^{MED}$ and $-\alpha^{4.2}$ deletions, nondeletional defects such as $\alpha_2^{Ncol}\alpha_1$, and the chain termination mutant Hb Constant Spring have been found in a few subjects. Two $(\alpha\alpha)^{thal}$ lesions have not yet been characterized. This aspect links Sicily with the Southern Italian Peninsula [Massa et al., 1994] and differentiates it from Sardinia where frequency of α-thal 2 is much higher (0.18 in the south and 0.13 in the north) and is marked by considerable molecular homogeneity: the $-\alpha^{3.7}$ is the prevalent mutation [Pirastu et al., 1982; Masala, 1992]. A probable explanation can be found in the historic isolation of the Sardinians, as compared to the large, constant migrations to Sicily from several Mediterranean countries (Greece, North Africa, Spain).

In Sicilian newborn infants, the γ-globin gene anomalies occur rather frequently [Huisman et al., 1991]. As is found in other countries of the Mediterranean Basin, the Hb $F^{Sardinia}$ variant is present everywhere. Furthermore, our data suggest the presence of different γ-globin gene arrangements, with a frequency that cannot be ignored. Sicily could reveal an unusual variety of mutations also in the γ fetal genes.

Spontaneous mutations, migrations, selective advantage of the heterozygote in an area that was at high risk for malaria, genetic drift, and the founder effect appear to be the main genetic factors for the origin of the high polymorphism of the globin system in the Sicilian population. Finally, the incidence of hemoglobinapathies seems to be highly influenced by the phenomenon of deviation of random mating, as has been demonstrated by the evaluation of the level of inbreeding, using the isonymy techniques [Crow and Mange, 1965; Schilirò et al., 1993].

The data presented in this study demonstrate how the frequency and the variety of the Hb anomalies represent a relevant social and health care problem that is being solved with a painstaking and efficient prevention program. This phenomenon does not exclusively affect our island. In fact, since the last 10 years of the 19th century, the high migration rates from Sicily to North and to South America, to Australia, and, finally, to the countries of northern Europe has made the Sicilian Hb variants of general interest.

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